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L-lysine dose dependently delays gastric emptying and increases intestinal fluid volume in humans and rats

Baruffol, C ; Jordi, J ; Camargo, S ; Radovic, T ; Herzog, B ; Fried, M ; Schwizer, W ; Verrey, F ; Lutz, T A ; Steingoetter, A

Abstract: BACKGROUND: Novel sensory inputs for the control of food intake and gastrointestinal (GI) function are of increasing interest due to the rapid increase in nutrition-related diseases. The essential amino acid L-lysine was demonstrated to have a selective impact on food intake, gastric emptying, and intestinal transit in rats, thus indicating a potential novel direct sensory input to assess dietary protein content and quality. The aim of this study was to assess translational aspects of this finding and to investigate the dose-dependent effect of L-lysine on human and rat GI function. **METHODS:** L-lysine doses from 0-800 mg in rats and 0.5-7.5 g in humans were analyzed for their effect on gastric emptying and GI secretion. Human GI function was assessed non-invasively using magnetic resonance imaging (MRI), rat data were acquired using standard lethal measurement methods. L-lysine dose dependently delayed gastric emptying and stimulated GI secretion in rats as reflected by residual phenol red content and increased gastric wet weight. **KEY RESULTS:** The dose-dependent delay in gastric emptying observed in rats was confirmed in humans with an increase in halftime of gastric emptying of 4 min/g L-lysine, $p < 0.01$. Moreover, a dose-dependent increase in intestinal fluid accumulation was observed (0.4 mL/min/g L-lysine, $p < 0.0001$). No effect on alkaline tide, glucose concentration, hematocrit, or visceral sensations was detected. **CONCLUSIONS INFERENCES:** This translational study demonstrates comparable dose-dependent effects of intragastric L-lysine on GI function in humans and rats and suggests a broader role for individual amino acids in the control of GI motility and secretion in vivo.

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L-lysine dose dependently delays gastric emptying and increases intestinal fluid volume in humans and rats

C. BARUFFOL,*,[#] J. JORDI,^{†,‡,§,¶} S. CAMARGO,^{‡,§} T. RADOVIC,*,[§] B. HERZOG,^{‡,§} M. FRIED,*,[§] W. SCHWIZER,*,[§] F. VERREY,^{‡,§} T. A. LUTZ,^{‡,§} & A. STEINGOETTER*

*Division of Gastroenterology and Hepatology, University of Zurich, Zurich, Switzerland

[†]Institute of Veterinary Physiology, University of Zurich, Zurich, Switzerland

[‡]Institute of Physiology, University of Zurich, Zurich, Switzerland

[§]Zurich Center for Integrative Human Physiology (ZIHP), University of Zurich, Zurich, Switzerland

Key Messages

Why this paper is important

- The essential amino acid L-lysine was demonstrated to have a selective impact on food intake, gastric emptying, and intestinal transit in rats, thus indicating a potential novel direct sensory input to assess dietary protein content and quality. This work represents an important translational extension of reported findings using magnetic resonance imaging (MRI) in human subjects and standard lethal measurements in rats. We show that intragastric L-lysine exhibits a comparable dose-dependent effect on GI motility and secretion in humans and rats. We strongly believe that these translational findings further support the broad role of specific amino acids in the control of GI function *in vivo*.

The aim of the research work

- The aim of this study was to assess the translational aspects of recent findings in rats demonstrating a selective impact of L-Lysine on gastrointestinal (GI) function and to investigate the dose-dependent effect of L-lysine on human and rat GI function.

Basic methodology of study

- L-lysine doses from 0–800 mg in rats and 0.5–7.5 g in humans were analyzed for their effect on gastric emptying and GI secretion. Human GI function was assessed non-invasively using MRI, rat data were acquired using standard lethal measurement methods.

Summarizing key results

- A dose-dependent delay in gastric emptying was observed in humans with an increase in halftime of gastric emptying of 4 min/g L-lysine, $p < 0.01$. A dose-dependent increase in intestinal fluid accumulation was observed (0.4 mL/min/g L-lysine, $p < 0.0001$).

Address for Correspondence

Andreas Steingoetter, Division of Gastroenterology and Hepatology, University of Zurich, Raemistrasse 100, Zurich CH-8091, Switzerland.

Tel: +41 44 255 56 84; fax: +41 44 255 45 03;

e-mail: steingoetter@biomed.ee.ethz.ch

[#]These authors contributed equally to this work.

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Abstract

Background Novel sensory inputs for the control of food intake and gastrointestinal (GI) function are of increasing interest due to the rapid increase in nutrition-related diseases. The essential amino acid L-lysine was demonstrated to have a selective impact on food intake, gastric emptying, and intestinal

transit in rats, thus indicating a potential novel direct sensory input to assess dietary protein content and quality. The aim of this study was to assess translational aspects of this finding and to investigate the dose-dependent effect of L-lysine on human and rat GI function. **Methods** L-lysine doses from 0–800 mg in rats and 0.5–7.5 g in humans were analyzed for their effect on gastric emptying and GI secretion. Human GI function was assessed non-invasively using magnetic resonance imaging (MRI), rat data were acquired using standard lethal measurement methods. L-lysine dose dependently delayed gastric emptying and stimulated GI secretion in rats as reflected by residual phenol red content and increased gastric wet weight. **Key Results** The dose-dependent delay in gastric emptying observed in rats was confirmed in humans with an increase in halftime of gastric emptying of 4 min/g L-lysine, $p < 0.01$. Moreover, a dose-dependent increase in intestinal fluid accumulation was observed (0.4 mL/min/g L-lysine, $p < 0.0001$). No effect on alkaline tide, glucose concentration, hematocrit, or visceral sensations was detected. **Conclusions & Inferences** This translational study demonstrates comparable dose-dependent effects of intragastric L-lysine on GI function in humans and rats and suggests a broader role for individual amino acids in the control of GI motility and secretion *in vivo*.

Keywords gastric emptying, gastrointestinal function, L-lysine, MRI.

INTRODUCTION

The worldwide rapid increase in nutrition and in particular obesity-related diseases stimulated efforts to understand how a meal impacts on various physiological processes including gastrointestinal (GI) function. Upon food intake, GI function adapts quickly to the ingested meal presumably to control efficient digestion and absorption of the vital nutrients. This dynamic plasticity is regulated by mechanisms responding to properties specific to the ingested meal such as their caloric content, volume, and consistency. This multiple signaling input is integrated to some extent by the autonomous nervous and endocrine systems to control proper GI function.¹

The macronutrient composition of a meal is commonly not considered to have a major impact on GI function.^{2,3} This is in contrast to newly emerging concepts demonstrating macronutrient-specific con-

trol of different vagal and endocrine signals. For instance, specific individual amino acids induce vagal afferent firing or stimulate the release of different GI hormones *ex vivo*.^{4–8} The *in vivo* relevance of these findings in the context of GI function control has typically been extrapolated from studies showing that high protein diets delayed gastric emptying to a larger extent than isocaloric control diets in both humans and rats.^{9,10} Controversially, others could not confirm these observations.¹¹ As high protein diets are composed of different individual amino acids and thereby considerably vary between laboratories, this might obscure amino acid-specific effects. So far, no study systematically tested the impact of all the 20 individual amino acids on GI function in humans or rats. Therefore, the selection of candidate amino acids was based on a recent study in rats revealing that L-arginine, L-lysine, and L-glutamic acid induced the most potent anorectic effect compared to isomolar doses of all other proteogenic amino acids.¹² Within this study, L-lysine was shown to delay solid gastric emptying, induce gastric secretion, and increase intestinal motility, thereby revealing an amino acid-specific impact on GI function *in vivo*. Several aspects remained elusive. Neither the dose-dependent effect nor the temporal dynamics of L-lysine were resolved, but both are relevant for the characterization of the pharmacokinetic effect of L-lysine. Most importantly, it remained unclear if humans show a similar GI response to a gastric load of L-lysine. Humans and rodents significantly differ in GI anatomy and physiology even though from an ingestive perspective both are omnivores. For instance, rats eat 10–20% of their bodyweight equivalent as food every day, whereas healthy humans at best 2%. Moreover, rats do not have a gallbladder and lack the vomiting reflex.

The aim of this study was to identify the dose-dependent effect of L-lysine on human and rat GI function after intragastric infusion or gavage, respectively. Animal experiments were restricted to the 30 min time point due to lethal methods. GI function was analyzed by measurements of wet weight and residual phenol red content. In humans, magnetic resonance imaging (MRI) and venous blood sampling were applied to repeatedly assess gastric meal, secretion, and intestinal fluid content volume as well as blood parameters every 15 min for over 90 min. Human experiments were performed in healthy volunteers within a randomized, double-blind, three-armed, cross-over study design. The study revealed a conserved impact of L-lysine on GI function in humans and rats.

MATERIALS AND METHODS

Ethical approval

All procedures for rat handling and experimental interventions were according to the Swiss Animal Welfare laws and approved by the Kantonales Veterinäramt Zürich. The human study was performed between April and September 2012 in a single center, randomized, double-blind, 3-armed, unbalanced, cross-over study design and in accordance with the Declaration of Helsinki, Good Clinical Practice. It was registered at ClinicalTrials.gov with identifier NCT01212614. The local Ethics Committee approved the study and all volunteers gave written informed consent.

Animal care

Thirty male Wistar rats (Janvier, Saint Berthevin Cedex, France) were grouped housed (room temperature 21 ± 1 °C, artificial 12/12 h light–dark cycle) with water and food (3433 Kliba Nafag, Kaiseraugst, Switzerland) available *ad libitum* unless noted otherwise.

Animal dose preparation

L-lysine of 0, 25, 50, 100, 200, and 400 mg/mL (Sigma-Aldrich, Buchs, Switzerland) was solubilized in tap water and 80, 75, 70, 60, and 40 mg/mL NaCl (Sigma-Aldrich) proportionally added to correct for molarity differences. pH was adjusted to 7.2 using HCl (Merck, Zug, Switzerland). The applied volume was 2 mL for each of these solutions supplemented with 1.5 mg phenol red (Sigma-Aldrich).

Animal experimental design

Animals were food deprived for 4 h prior oral L-lysine administration to ensure similar residual stomach content. Rats received intragastric application of 2 mL of the respective L-lysine dose and were returned to their home cage, where they did not have access to food or water. Thirty minutes postapplication, animals were euthanized using pentobarbital (IP, 100 mg/kg, Kantonsapotheke Zürich, Switzerland) in combination with isoflurane (5%) for quicker induction. The GI tract was excised and segmented into stomach, small intestine, cecum, and colon based on anatomical landmarks using clamps to limit liquid loss. The wet weight of each segment was immediately measured. Phenol red was extracted from each segment and quantified by optical spectroscopy at 560 nm wavelength as described previously.¹²

Animal Statistics

Rats were randomly allocated into the six treatment groups. Application order was randomized and statistical analysis performed as described for the human data set below.

Human Subjects

Six healthy subjects aged between 18 and 50 years (four male, two female, BMI 22 ± 1 kg/m²) were recruited via a public advertisement. A *T*-test-based power analysis was performed based on data from a previous study in 14 healthy subjects that measured the

production rate of gastric secretion after intake of a secretion stimulating test meal.¹³ A statistical power of 0.83 was determined for the inclusion of six subjects, an effect size of 1.5 and a significance level of 0.05. Prior study inclusion, all subjects underwent a clinical assessment and were tested negative for *helicobacter pylori*. The following exclusion criteria were applied: BMI (kg/m²) <19 or >24, history of systemic and psychiatric disease especially GI disease or surgery (excludes appendectomy, hernia repair, and anorectal disorders), lysineuria, galactosaemia, medication intake of any kind, drug or alcohol abuse, presence of metallic implants, devices or metallic foreign bodies, claustrophobia, pregnancy and lactation, known allergy or intolerance against locus bean gum, L-lysine, Gadolinium (DOTAREM®), Methylparaben (E218), Fructose, Gluten, Galactose, and also allergic reaction after prior injection of Lidocain (e.g., at the dentist).

Human test meal

L-lysine was administered as L-lysine monohydrate (LMH, Fagron GmbH & Co.KG, Barsbüttel, Germany) at doses of 0.5, 1.2, 3.0, and 7.5 g in four different 300 mL test meals. The maximum L-lysine dose of 7.5 g was selected based on literature data. Doses up to 9 g are considered a physiological daily dose,¹⁴ however, doses >8 g have been reported to induce diarrhea and cramps.¹⁵ Lower doses were derived using a reduction factor of 2.5. The ingredients and properties of the four LMH test meals had negligible caloric content (<25 kcal) and are listed in Table 1. NaCl was proportionally added to each test meal to correct for molarity differences. All meals were prepared directly before each study session, heated to 37 °C, and infused within 1–2 min while volunteers were lying in the MRI scanner.

Human measurement protocol

The human dose–response study was performed using a randomized, double-blind, 3-armed, unbalanced, cross-over study design. Three of the four L-lysine test meals were randomly assigned to the six subjects. This unbalanced study design was selected to receive maximum output while minimizing exposure. All six subjects underwent three study sessions of 2.5 h including subject preparation and repeated MRI and venous blood measurements. Sessions were performed on three different days separated by 5–21 days. Each session followed the same procedure depicted in Fig. 1. Subjects arrived at the MR center in the morning or at noon after fasting overnight (or for at least 8 h). A nasogastric tube (Freka® Tube CH/FR 12) was

Table 1 Ingredients and properties of the four L-lysine test meals for human application.

Ingredients/properties (units)	Values			
LMH (g)	0.5	1.2	3.0	7.5
NaCl (g)	1.24	1.12	0.8	0.0
Evian® (still water; mL)	300			
Locus bean gum (E410; g)	3			
MR contrast agent (Dotarem®, µL)	350 (± 175 µmol ± 583.3 µM)			
Osmolarity (mOsm/L)	152.52			
pH	9.2	9.6	9.6	9.85
Caloric load (kcal)	2.3	4.5	10.4	25.1

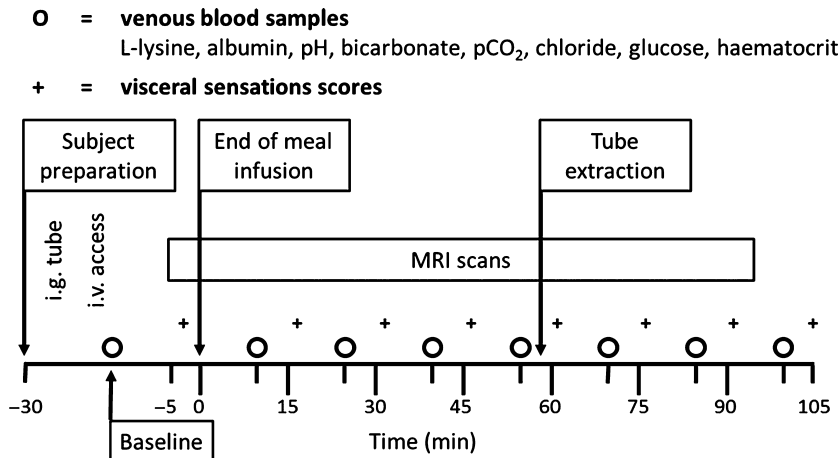


Figure 1 Schematic of the human study protocol. The schematic depicts the sequence of MRI measurements, venous blood sampling, and visceral sensation ratings as performed in all three study sessions.

placed 50–60 cm from the nares after previous local anesthesia of the nasal cavity with Lidocain-HCl 2% (prepared in house) and Vibrocil® (Novartis, Basel, Switzerland) nose gel. A venous vascular cannula (Vasofix® Braunüle®) was placed on the right or left cubital vein for subsequent blood sampling and continuous 0.9% NaCl infusion. MR image data were acquired with subjects positioned in right decubitus before and directly after test meal infusion and then every 15 min until 90 min. Visceral sensations scores were recorded after each MRI scan and in addition at 105 min after meal infusion. Blood samples were collected before (baseline) and at 10, 25, 40, 55, 70, 85, and 100 after meal infusion. The first blood sample in fasted state and last blood samples at 100 min were taken in sitting position, all other samples were obtained while the subject was lying on the MR patient table. The nasogastric tube was removed at ~60 min with subjects in sitting position.

Human MRI and visceral sensations scores

Subjects were positioned in right decubitus inside the clinical MRI scanner (1.5 T Achieva, Philips Healthcare, Best, The Netherlands). An abdominal phased-array surface coil (SENSE body coil, four elements) was used for image acquisition. MR imaging consisted of three imaging sequences to visualize gastric content volume, gastric secretion, gallbladder volume, and intestinal fluid content. The MRI scan parameters for gastric content volume measurement were as follows: balanced steady-state free precession sequence, 26–34 transverse slices, 6 mm slice thickness, no slice gap, 360 mm field of view, 1.6/3.3 ms echo/repetition time, one breath hold, 16–24 s scan duration (depending on slice number). For assessing gastric secretion, MRI scan parameters were as follows: radial T1-weighted gradient echo sequence with profiles acquired in golden angle view order, 18 coronal slices, 6 mm slice thickness, 0.6 mm slice gap, 360 mm field of view, 1.8/12 ms echo/repetition time, free breathing, 2:24 min scan duration. To visualize gallbladder volume and intestinal fluid content, MRI scan parameters were as follows: T2-weighted turbo spin echo sequence with fat suppressions, 26 coronal slices, 5 mm slice thickness, 1 mm slice gap, 375 mm field of view, 80/1200 ms echo/repetition time, two breath holds, 31 s scan duration.

After MRI scans, subjects had to report their visceral sensations, i.e. hunger, fullness, nausea, bloating, abdominal cramps, and urge to defecate using a self-assessed sensations scale ranging from 0 to 10 (0 – no sensations, 10 – extremely prominent sensations).

Human blood sampling and analysis

For blood gas measurements assessing the alkaline tide (pH, bicarbonate, pCO₂, chloride), glucose and hematocrit, venous blood was first collected with a serum vacutainer, then filled within 1–3 min in heparinized capillaries, and analyzed using the ABL 700 blood gas analyzer (Radiometer GmbH, Thalwil). For measurements of albumin and L-lysine blood concentration, Li-heparin vacutainers and EDTA-vacutainers were used, respectively. Albumin was measured to assess a potential shift in fluid from intravascular to intragastric. Blood samples were directly centrifuged and ~1 mL of plasma was pipetted for storage at –20 °C for L-lysine and 8 °C for albumin. A volume of 50 µL of each L-lysine plasma sample was mixed with 50 µL of 10% SSA/NVal solution and centrifuged at 22639.5 g for 5 min. A volume of 60 µL borate/NaOH buffer and 20 µL reagent (2AMT) was added to 20 µL of the resulting supernatant and 1/100 µL of this mixture was injected on a HPLC column using the cell culture gradient, according to the manufacture's protocol. The plasma for the albumin measurement was processed with a Cobas 8000 modular analyzer (c701 module, Roche, Mannheim) subsequent a color test method (Bromocresol green) with endpoint measure.

Data analysis

Magnetic resonance image analysis was performed blindly by a radiologist in training (TR) and a research assistant with 1 year of training in analyzing abdominal MR image data (CB). Purpose built software tools implemented in MATLAB (The MathWorks, Natick, MA, USA) and IDL (Exelis Visual Information Solutions) were applied for extraction of gastric content volume, gastric secretion, gallbladder volume, and intestinal fluid content. Gastric content contours were segmented in each transverse image by a semi-automated algorithm. Respective 3D gastric content volumes were computed from 3D isosurfaces of the contours.¹³ Gallbladder contours and small intestinal areas excluding blood vessels and visceral tissues were manually contoured in each T2-weighted coronal image slice. To assess intestinal fluid content, a signal threshold was selected to segment the bright luminal fluid content from air and surrounding soft tissue. Intestinal fluid volume was calculated by summing all segmented pixels. Gastric secretion volume was analyzed from reconstructed T1 maps according to a previously established analysis method.¹³

Magnetic resonance parameters were smoothed using function *loess* (with smoothing parameter $\alpha = 1$) in program R¹⁶ and plotted over time and LMH dose. Halftimes of gastric emptying (t_{50}) were calculated from smoothed curves by solving the locally smoothed interpolation for 50% using the Newton method.¹⁷

Dose response of t_{50} was analyzed by linear mixed model with t_{50} and LMH dose as fixed effects and subject as random effect. Dose response of gallbladder and intestinal fluid volumes was analyzed by linear mixed model with volume, time, and LMH dose as fixed effects and subject as random effect.

The density functions of individual mean visceral sensations scores were plotted. As the resulting distributions were dominated by zero values, visceral sensations scores were divided into two categories, i.e., 0 and ≥ 1 , to only test for a dose-dependent increase in scores not zero. The dose-dependent effect of L-lysine on the binomial visceral sensations scores was analyzed using generalized linear models with a binominal link function.

Deltas over baseline (DOB) values were calculated for L-lysine and albumin concentrations, all blood gas parameters, and base excess. Baseline was defined as the values detected at fasted state. DOB values were smoothed using function *loess* and plotted over time and LMH dose. Dose response was analyzed by linear mixed models with DOB of blood gas parameters, time, and LMH dose as fixed effects and subject as random effect. Estimated model parameters are presented as estimate \pm standard error. A significance level of 0.05 was chosen.

RESULTS

Animal experiments

To identify a dose-dependent impact of L-lysine on rat GI function, we administered different isomolar doses of L-lysine intragastrically and measured GI tract wet weight and phenol red transit 30 min post-application. The doses applied ranged from 0 to 800 mg L-lysine per animal covering the span of daily L-lysine intake in rats.¹² Rat stomach and cecum wet weight dose dependently increased by 0.4 ± 0.04 g/0.1 g L-lysine ($p < 0.0001$) and 0.2 ± 0.01 g/0.1 g L-lysine ($p = 0.03$), respectively (Fig. 2A and B). No other GI segments showed a dose-dependent change in wet weight. As animals did not have access to water or food, we

interpreted these wet weights changes to reflect alterations in secretion or absorption, respectively. To assess GI motility more specifically, we tracked the transit of a dye (phenol red) along the GI segments 30 min postapplication. By measuring residual phenol red in the stomach, we observed delayed gastric emptying by increased gastric phenol red content of $3.0 \pm 0.8\%$ per 0.1 g L-lysine ($p < 0.001$) and decreased small intestinal phenol red content of also $3.0 \pm 0.4\%$ per 0.1 g L-lysine ($p < 0.0001$, Fig. 3A and B). In accordance, colon phenol red content decreased by $0.3 \pm 0.08\%$ per 0.1 g L-lysine ($p < 0.001$, Fig. 3C). The gastric and intestinal phenol red content appeared to plateau at the two higher L-lysine doses. These data show that L-lysine dose dependently delayed gastric emptying and stimulated gastric secretion in rats. In addition, cecum wet weight increased dose dependently indicating alterations in intestinal, pancreatic, or biliary secretion. This data is limited to the 30 min time point, thereby potentially obscuring lower dose effects of L-lysine.

Human experiments

To assess the translational aspects of L-lysine impact on GI function, we conducted a MRI based study in healthy human subjects. Briefly, we administered different isomolar doses of L-lysine nasogastrically and measured the impact on GI function and blood gas parameters every 15 min over a time course of 90 min. The administered doses were between 0.5 and 7.5 g L-lysine per test meal, which is considered physiological relevant as the daily L-lysine intake varies between 2 and 9 g/day.¹⁴ In one subject, L-lysine measurements for one dose had to be dismissed due to non-compliance with study procedure (subject P4, 3 g L-lysine dose). After L-lysine application, blood L-lysine concentration increased dose dependently

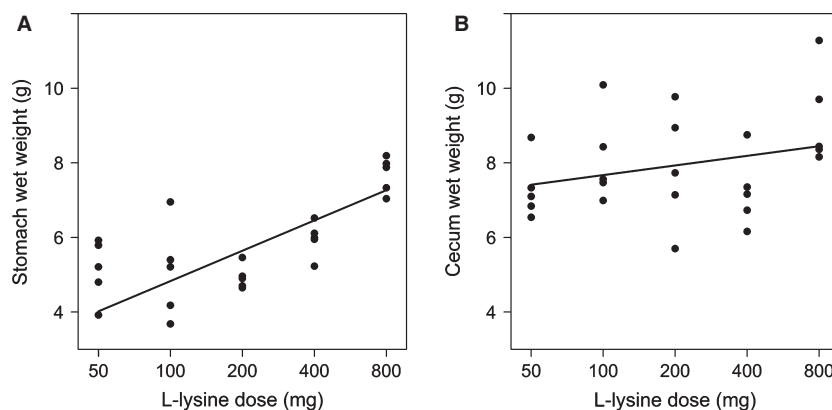


Figure 2 Dose-dependent effect of intragastric L-lysine administration on GI wet weight in rats. L-lysine dose dependently increased stomach (A) and cecum wet weight (B) in rats. Individual points represent wet weight measurements of individual animals treated with the respective L-lysine dose. The predicted linear dose-response curve is presented as solid line. L-lysine doses (x-axis) are plotted in log₂ scale.

over time by $0.54 \pm 0.1 \mu\text{M}/\text{min}/\text{g}$ L-lysine ($p < 0.0001$, Fig. 4A and B) demonstrating proper application and absorption. No dose-dependent differences were found for albumin or any of the blood parameters. At the level of the stomach, L-lysine dose dependently delayed gastric emptying by an increase in t_{50} of $4.2 \pm 1.4 \text{ min}/\text{g}$ L-lysine ($p = 0.01$, Fig. 5A and B). The infused test meals with 0.5 g and 1.2 g L-lysine emptied similar to a drink of 300 mL of water.¹⁸ No changes in gallbladder volume or gastric secretion were detected based on MRI. In the small intestine, L-lysine dose- and time-dependently increased fluid volume by

$0.37 \pm 0.1 \text{ mL}/\text{min}/\text{g}$ L-lysine ($p < 0.0001$, Fig. 6A and B). This effect and its dynamics were clearly detectable in the MR image data (Fig. 7). During the 100 min postprandial period, no side effects or dose-dependent changes in self-reported visceral sensations were found. After end of study protocol, defecation was altered after the intake of 7.5 g L-lysine, as four of five subjects receiving this highest dose retrospectively reported self-limiting diarrhea within 1–6 h after L-lysine application. No such change in defecation was reported for any other dose. In summary, these findings confirm the dose-dependent delay in gastric

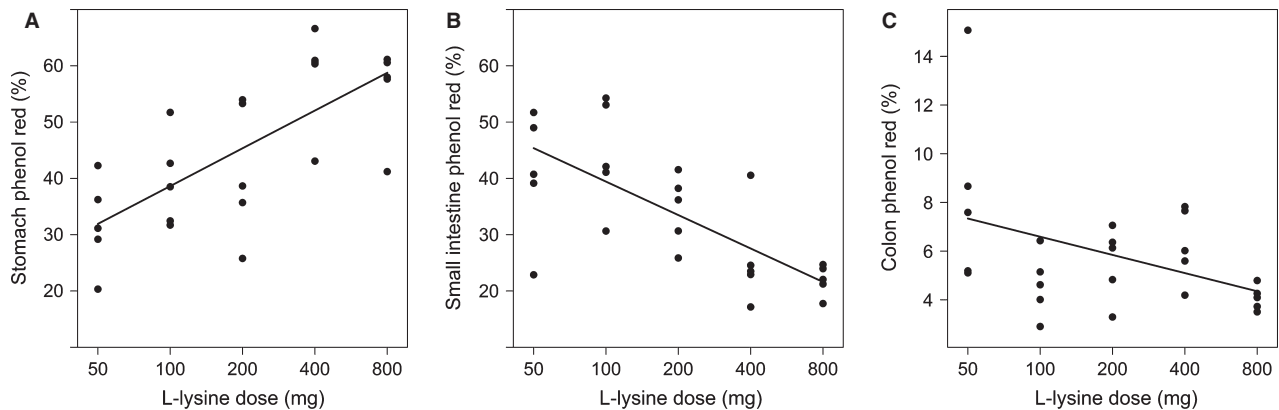


Figure 3 Dose-dependent effect of intragastric L-lysine administration on phenol red content in rats. Phenol red content is expressed as percentage of the initially administered total phenol red amount. L-lysine dose dependently increased gastric phenol red content (A) and decreased small intestinal (B) and colon (C) phenol red content in rats. Individual points represent residual phenol red content measurements of individual animals treated with the respective L-lysine dose. The predicted linear dose-response curve is presented as solid line. L-lysine doses (x-axis) are plotted in log_{2.0} scale.

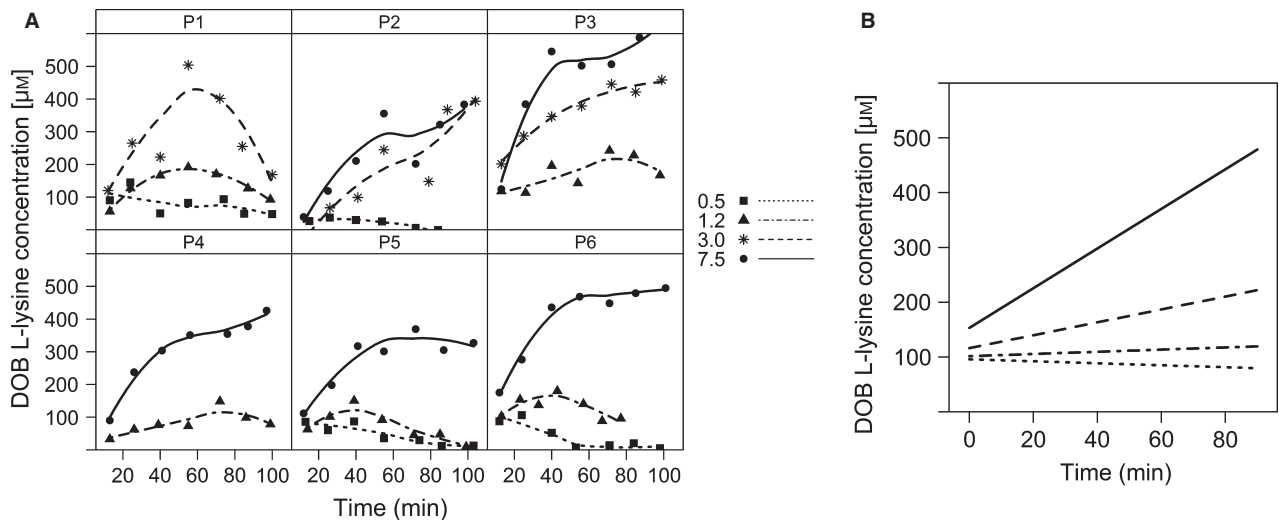


Figure 4 Dose-dependent L-lysine concentrations in venous blood after intragastric application of L-lysine in humans. Data are expressed as delta over baseline (DOB) with baseline values measured before intragastric L-lysine infusion. (A) Smoothed DOB L-lysine concentration curves (lines) overlaid on the individual measurements (points) of all human subjects, each treated with three (of the total four) different L-lysine doses. L-lysine concentration data of one dose in subject P4 were dismissed due to non-compliance of study protocol. (B) The average linearly predicted L-lysine concentration increase per dose.

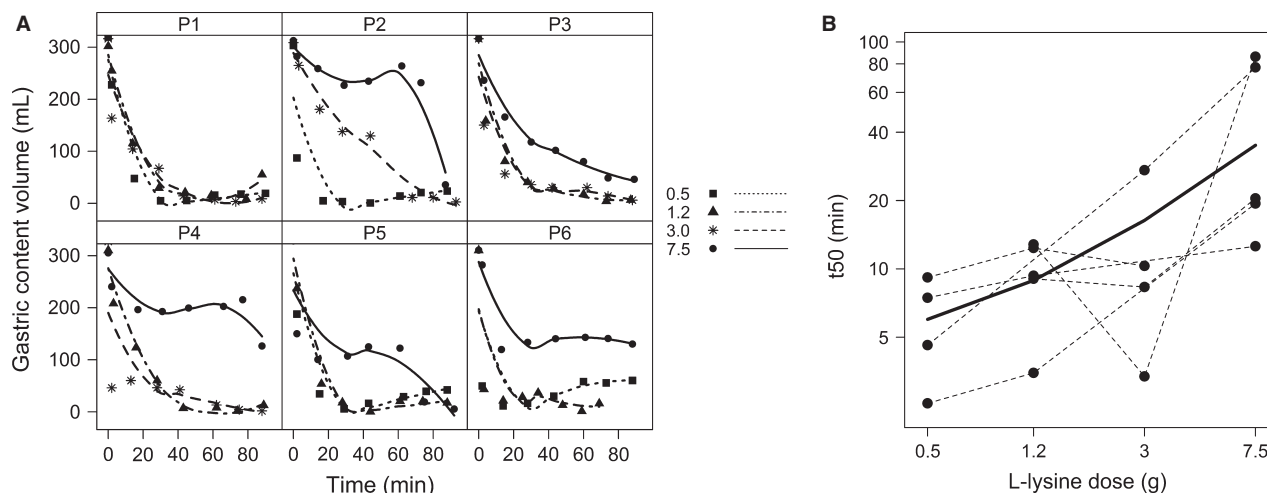


Figure 5 Dose-dependent effect of intragastric L-lysine application on gastric emptying in humans. (A) Smoothed gastric emptying curves (lines) overlaid on the individual volume measurements (points) of all human subjects, each treated with three (of the total four) different L-lysine doses. (B) L-lysine dose dependently increased t50 (computed from smoothed gastric emptying curves) in humans. The predicted linear increase is indicated as solid line. Values of t50 (y-axis) are plotted in log10 scale, L-lysine doses (x-axis) are plotted in log2.5 scale.

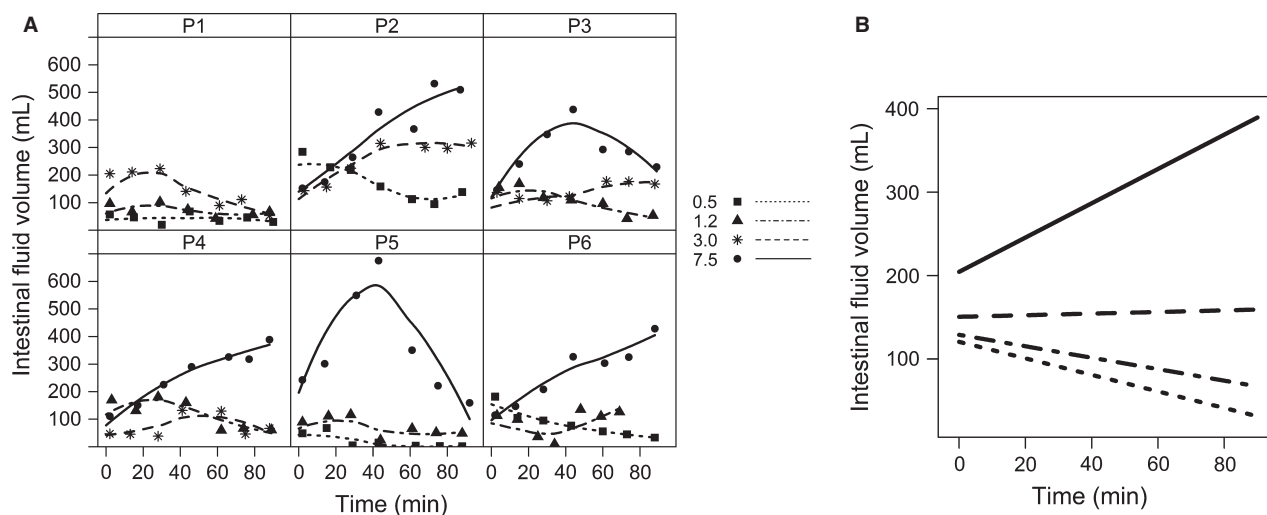


Figure 6 Dose-dependent effect of intragastric L-lysine application on intestinal fluid volume in humans. (A) Smoothed intestinal fluid volume curves (lines) overlaid on the individual volume measurements (points) of all human subjects, each treated with three (of the total four) different L-lysine doses. (B) The average linearly predicted intestinal fluid volume change per dose.

emptying and demonstrate an increase in intestinal liquid content in humans.

DISCUSSION

This study revealed several similar functional responses of the GI tract to L-lysine stimulus in humans and rats. In both species, gastric and small intestinal functions were effectively altered after intragastric infusion of L-lysine.

L-lysine delayed gastric emptying revealing a conserved amino acid-specific effect in rats and humans. This delay seemed not to trigger a temporal delay of

L-lysine absorption into the blood stream in humans for enabling postgastric regulatory mechanisms. Gastric emptying is generally accepted to be primarily controlled by the volume and the caloric equivalent of the gastric load.¹ The two low-dose test meals (0.5 and 1.2 g) emptied faster or similar to a 300 mL drink of water,¹⁸ while the highest 7.5 g L-lysine dose delayed the volume emptying by ~30 min. The negligible caloric differences between the lowest and highest L-lysine doses in humans (<23 kcal) and also rats (<5 kcal) suggest a caloric-independent mechanism for the observed alterations in gastric emptying. This amino acid-specific mechanism for the control of GI

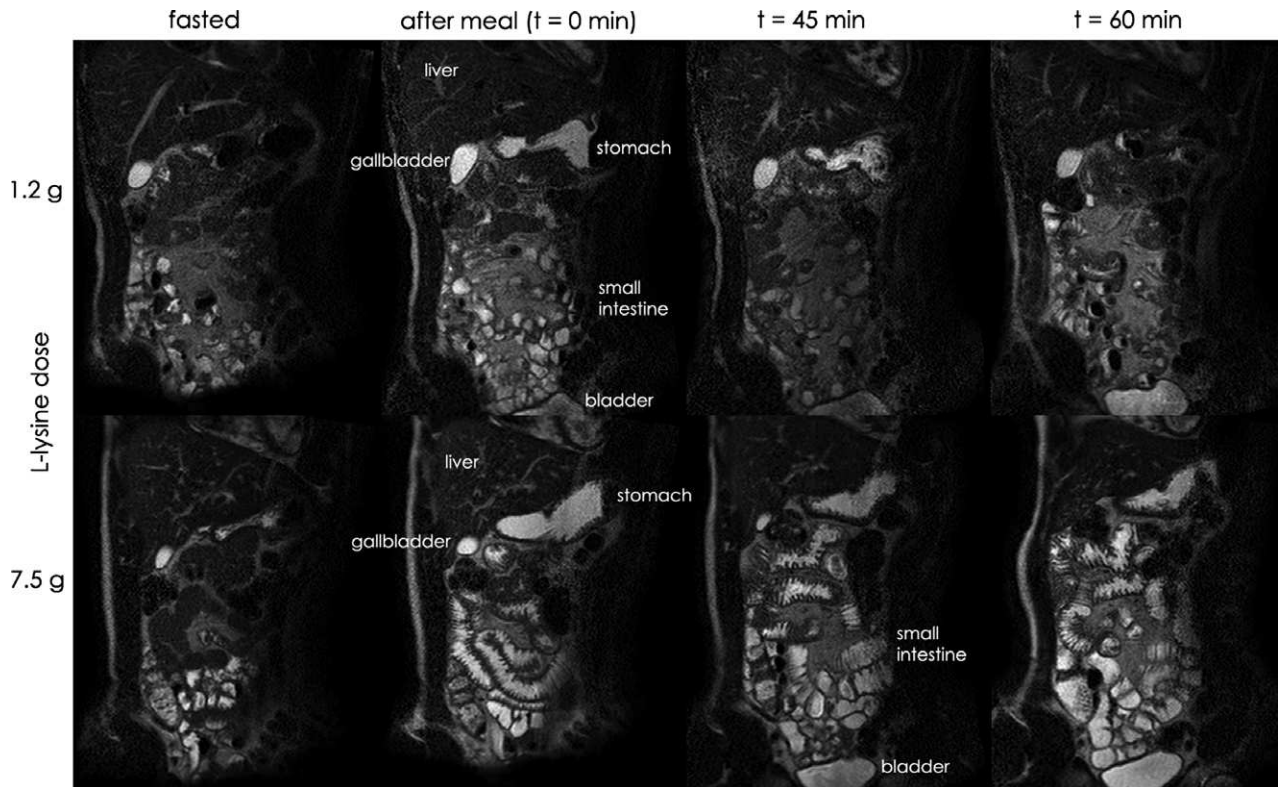


Figure 7 MR image data highlighting the dose-dependent effect of intragastric L-lysine application on gastrointestinal fluid volume in humans. The difference in the accumulation of intestinal fluid volume and the delay in gastric emptying between L-lysine doses 1.2 and 7.5 g is depicted over 60 min in representative MR image data of one subject (P6). Imaged GI organs and anatomical landmarks are indicated.

function has been recently proposed for L-lysine, L-arginine, and L-glutamic acid in rats¹² and is here-with confirmed for humans. Beyond that, the former rat study showed that intravenous L-arginine and L-lysine did not modulate GI function indicating a potential GI luminal mechanism of action.¹²

This work's focus was limited to the short-term effect of L-lysine on GI motor function in rats and humans. Our previous work in rats did not suggest a long-term effect, as L-lysine reduced food intake only in the first hour after application, but did not alter energy intake in the subsequent hours.¹⁹ On the basis of this anorectic effect, we concluded that L-lysine acts in the short term rather than in the long term. No simultaneous measurements of enteroendocrine hormones were performed that would have allowed to gain further insights with regard to the mechanism or site of action. Indeed, L-lysine might have stimulated the release of specific GI hormones. For instance, the release of the gut hormone GLP-1 from primary murine intestinal cells was shown to be induced by L-glutamic acids.²⁰ Similar L-phenylalanine and L-tryptophan were reported to stimulate cholecystokinin (CCK)

secretion from primary cells.²¹ These findings demonstrated the remarkable amino acid specificity for the secretory function of endocrine cells *ex vivo*. However, the specific impact of L-lysine on GI hormone release and their relevance for the modulation of GI function *in vivo* are currently unknown. The underlying regulatory mechanism of the L-lysine effect might have also involved vagal signaling. Several *ex vivo* electrophysiological studies revealed amino acid specificity of vagal firing as different amino acids excite or inhibit vagal afferent activity dependent on the exposed vagal branch.^{4–8} Interestingly, of all amino acids tested, only intragastric L-glutamic acid induced vagal signaling *ex vivo* indicating the necessity for amino acid absorption.^{22,23} Hepatic vagal afferents fire specifically to an L-lysine stimulus and they were shown to mediate the anorectic effect of L-lysine in rats.^{8,12} Taken together, this study showed that L-lysine dose dependently delayed gastric emptying suggesting a caloric-independent mechanism for the control of GI function also in humans.

Gastric secretion was dose dependently induced in animals, but not in humans. Importantly, delayed

gastric emptying and secretion had a different dose-response curve in rats. Hence, the L-lysine doses used here in humans might have been insufficient to extensively stimulate gastric secretion. A formerly more invasive study by Sackler *et al.* applying repeated extraction of gastric content with subsequent titration reported increased gastric pepsin and HCl secretion after application of 5-g L-lysine combined with a solid test meal.²⁴ Hence, the test meal composition and the sampling method might have synergized with L-lysine to induce increased gastric secretion. It is understood that gastric secretion is mainly mediated by parietal cells. Here, we did not assess if L-lysine stimulated parietal secretion directly or induced histamine secretion by enterochromaffin cells; however, because histamine not only induces gastric secretion but also delays gastric emptying this might be an alternative mediatory mechanism of the observed L-lysine action.^{25,26} The lack of L-lysine's effect on gastric secretion in humans was reflected and supported by the absence of any differences in blood gas parameters and base excess. Gastric secretion was previously shown to correlate with base excess in blood, an effect termed alkaline tide.²⁷ Taken together, L-lysine induced gastric secretion at high doses in rats, and a similar effect might be probable in humans. However, its analysis is hampered by the diarrhea inducing side effect of L-lysine doses ≥ 7 g. The reported side effects of self-limiting diarrhea for the highest L-lysine dose of 7.5 g in humans were accompanied by an increase in intestinal fluid volume. In both humans and rats, a dose-dependent increase in intestinal fluid volume, respectively cecum wet weight, was observed. The origin of this fluid accumulation remains currently unclear, but might arise either from increased bile and pancreatic secretions, reduced luminal absorption, and/or active intestinal fluid influx. A simple osmotic effect seems unlikely, because we applied isomolar L-lysine solutions. As no dose-dependent effect on gallbladder volume was detected in humans, and because rats have no gallbladder, the latter two possibilities are currently the more likely reasons for this phenomenon.

In healthy humans, the apparent impact of L-lysine on gastric and small intestinal function did not translate into changes in visceral sensations scores. Hunger and fullness were the only scores to exhibit density functions with modes different from zero. Therefore, a simplified ordinal, i.e. binomial, model analysis had to be applied to test for a dose-dependent increase in scores ≥ 1 . This finding might not be surprising given the low numbers of study participants and the multitude of determinants of visceral sensitivity.²⁸ A larger number of volunteers would be

needed to conclude on the effect of L-lysine on human satiation.

The here presented work focused on liquid emptying. This may be regarded as methodological simplification in so far as animals and humans normally ingest solid and semi-solid meals, respectively. However, it is important to acknowledge that after a so-called 'lag phase', solid meals empty at a similar rate as liquid meals of similar composition and energy content.²⁹ Within this initial phase of gastric emptying, secretion and motor activity process the ingested food into a liquid-like dispersion that can pass through the pyloric opening. Hence, the focus on liquid emptying is also reasonable from a physiological perspective.^{30,31} This is also supported by previous data in rats showing delayed solid gastric emptying at a L-lysine dose of 6.7 mmol/kg.¹⁹ Within the human setting, liquid gastric emptying has become of more interest also in the clinical setting and is currently evaluated as a method for the detection of gastroparesis.³²⁻³⁵ In conclusion, this translational study demonstrates comparable dose-dependent effects of intragastric L-lysine on GI function in humans and rats, in particular stomach and small intestinal function. This conserved chemospecificity supports a broader role for individual amino acids in the control of GI transport and secretion *in vivo*.

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DISCLOSURE

All authors declare no competing financial, personal, or professional interest that could be construed to have influenced this paper.

AUTHOR CONTRIBUTION

All authors contributed to study design, data interpretation, and manuscript revision. CB performed all human experiments with the help of TR and AS; JJ performed all rat experiments with the help of BH; JJ and CB wrote the paper with the help of FV, TAL, and AS. All aspects of the project were supervised by FV, TAL, and AS. All authors contributed to manuscript editing and approved the final version of the manuscript. All authors qualifying for authorship are listed.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Albumin concentration and blood gas parameters from venous blood after intragastric L-lysine application in humans.

Figure S2. Visceral sensations scores after intragastric L-lysine application in humans.